PB82-239039

Changes in the Free Amino Acid Pool during Environmental Stress in the Gill Tissue of the Oyster, 'Crassostrea virginica'

Texas A and M Univ., College Station

Prepared for

Environmental Research Lab.
Gulf Breeze, FL

30 Jul 81

U.S. Department of Commerce
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TECHNICAL REPORT DATA
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1. REPORT NO.	2.	3. R
EPA-600/J-82-213	Journal Article	PB82-239039

4. TITLE AND SUBTITLE	5. REPORT DATE
CHANGES IN THE FREE AMINO ACID POOL DURING ENVIRONMENTAL STRESS IN THE GILL TISSUE OF THE OYSTER, CRASSOSTREA VIRGINICA	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
E.N. Powell, M. Kasschau, E. Chen, M. Koenig, and J. Pecon	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Department of Oceanography Texas A&M University College Station, TX 77843	11. CONTRACT/GRANT NO. R806793-01

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
U.S. Environmental Protection Agency Environmental Research Laboratory Office of Research and Development Gulf Breeze, FL 32561	14. SPONSORING AGENCY CODE EPA/600-J-

15. SUPPLEMENTARY NOTES
Ref: Comp. Biochem. Physiol. 71A(4):591-598. 1982.

16. ABSTRACT

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3. After 2 days, both oysters exposed to turbidity and to drilling effluents had increasee cysteic acid levels. Glutamic acid and alanine levels were also elevated in oysters exposed to drilling effluents.

4. After 5 days, glycine, alanine and β-alanine remained above control levels in oysters exposed to increased salinity whereas in those expsed to anoxia, turbidity and drilling effluents, a significant decrease in most amino acids occurred with the total FAA pool decreasing by 50%.

5. The FAA pool's response was unique for each stress studied suggesting that the FAA pool may prove to be a useful diagnostic tool for determining *a posteriori* the causative agent responsible for a given stress response.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to public	unclassified	10
	20. SECURITY CLASS (This page)	22. PRICE
	unclassified	

REPRODUCED BY
NATIONAL TECHNICAL INFORMATION SERVICE
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SPRINGFIELD, VA 22161

EPA-600/J-82-213
JOURNAL ARTICLE

Comp. Biochem. Physiol. Vol. 71A, No. 4, pp. 591 to 598, 1982
Printed in Great Britain

0300-9629/82/040591-08$03.00/0
Pergamon Press Ltd

CHANGES IN THE FREE AMINO ACID POOL DURING ENVIRONMENTAL STRESS IN THE GILL TISSUE OF THE OYSTER, *CRASSOSTREA VIRGINICA*

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(*Received* 30 *July* 1981)

Abstract—1. Oysters were exposed for 2- and 5-day periods to increased salinity ($26‰$–$38‰$), anoxia, turbidity and drilling effluents.

2. After two days, the FAA pool in the gill tissue of oysters exposed to $38‰$ salinity had elevated glycine, alanine and β-alanine levels; oysters exposed to anoxia showed elevated glycine and alanine and decreased aspartic acid levels.

3. After 2 days, both oysters exposed to turbidity and to drilling effluents had increased cysteic acid levels. Glutamic acid and alanine levels were also elevated in oysters exposed to drilling effluents.

4. After 5 days, glycine, alanine and β-alanine remained above control levels in oysters exposed to increased salinity whereas in those exposed to anoxia, turbidity and drilling effluents, a significant decrease in most amino acids occurred with the total FAA pool decreasing by 50%.

5. The FAA pool's response was unique for each stress studied suggesting that the FAA pool may prove to be a useful diagnostic tool for determining *a posteriori* the causative agent responsible for a given stress response.

INTRODUCTION

The free amino acid (FAA) pool of marine invertebrates is a center of metabolic activity during a variety of stress response phenomena. These include hyper- and hypo-osmotic stress induced by changes in salinity (e.g. Gilles, 1972; Weber & van Marrewijk, 1972; Hoyaux *et al.*, 1976; Baginsky & Pierce, 1978), anoxic stress (e.g. Felbeck, 1980; Warren & Dales, 1980; de Zwaan & Wijsman, 1976; de Zwaan *et al.*, 1976), various biological interactions such as parasitism (Feng *et al.*, 1970; Mack *et al.*, 1979) and starvation (Squires *et al.*, 1979), and a variety of manmade pollutants (e.g. Jeffries, 1972; Roesijadi & Anderson, 1979; Kasschau *et al.*, 1980). Most work has concentrated on the changes in various components of the FAA pool under a single stress condition. Clear and predictable changes are known to occur in many euryhaline species during salinity stress, for example. In addition, a number of recent studies suggest that two stresses presented concurrently might superimpose two distinct metabolic phenomena, though possibly modifying each to some extent, resulting in an FAA pattern different from that produced by either one alone (Roesijadi *et al.*, 1976; Kasschau, 1975a).

Since most specific stress phenomena seem to initiate specific metabolic responses in the FAA pool, the amino acid pool might be used as an effective monitoring agent for physiological condition in many invertebrates much as blood chemistry is used in humans. Attempts have been made to use a glycine/taurine ratio, for example (Jeffries, 1972) or the entire FAA pool (Sansone *et al.*, 1978). Although such a strategy has not been extraordinarily successful primarily due to the limited understanding of amino acid metabolism in the invertebrates at the present time, the number of amino acids that can be assayed for relatively quickly and the manifold pathways and control systems governing the FAA pool's size and composition certainly suggest that their potential for monitoring physiological condition is high.

The purpose of this study was to examine the FAA pool of the oyster *Crassostrea virginica*, during exposure to four stress-inducing agents to determine whether the amino acid pool might be used to identify agents inducing stress and to monitor stress phenomena. Three natural stresses were used: (1) hyperosmotic stress induced by a salinity increase and (2) anoxia—both of which are known to cause specific metabolic changes in the FAA pool (Lynch & Wood, 1966; Collicutt & Hochachka, 1977)—and (3) turbidity, which is known to deleteriously affect the filtering capability of many bivalves (Loosanoff & Tommers, 1948; Loosanoff, 1962). The fourth stressing agent was drilling effluents which are by-products of oil drilling operations (Thompson & Bright, 1977). Drilling effluents combine the stress of turbidity with a number of noxious chemicals including biocides and heavy metals and have been shown to be harmful to a variety of invertebrates (Krone & Biggs, 1980; Cantelmo *et al.*, 1979; Tagatz *et al.*, 1979; Thompson, 1980). We chose to monitor oyster gill tissue since this tissue is intimately involved in filtration and, therefore, potentially most important in adjusting to the stress of increased turbidity. We have examined the free amino acid pattern after 2 and 5 days under these conditions to determine whether such diverse stresses as used here could be effectively distinguished using

the FAA pattern without prior knowledge of the stressing agent involved. This would be the first step in establishing a diagnostic technique for physiological condition based on the FAA pool.

MATERIALS AND METHODS

Oysters were collected from the south jetty at Aransas Inlet, Texas. They were all small (about 2–4 cm long) and were collected at a moderately low salinity for this area—26‰. Salinities normally fluctuate substantially in the inlet over the year (≃26–36‰, Brown *et al.*, 1976) and turbidity is usually quite high. Visibility was less than 10 cm on the day of collection.

Oysters were transported to the laboratory and placed in 1 l. vessels, four to five per vessel. Seawater (26‰) collected from Aransas Inlet was used in all experiments. The water was constantly stirred and aerated and changed completely at least twice per day. After a 2-day acclimation period, the following experimental conditions were established:

(1) controls at 26‰; (2) salinity-stressed at 38‰—salinity was increased by the addition of Instant Ocean; (3) anoxia—N_2 was bubbled constantly through the water so that active filtration could occur even during anoxia; (4) turbidity—kaolin, which had been dialyzed exhaustively against 26‰ seawater was added to yield a concentration of 0.326 gm/l (w/v); (5) drilling effluent was added to yield a final concentration of 4 gm/l (v/v)—a particulate load of 0.042 g dry weight/g fluid.

Kaolin concentration was roughly three times the level Loosanoff (1962) found to induce changes in filtration rate in oysters and the drilling effluent about 1.5 times the equivalent kaolin concentration inducing changes in pumping rate (Loosanoff, 1962). It was noted soon after the experiment began that the kaolin, and to a much lesser extent the drilling effluent particulates, were removed rapidly from solution by the oysters. To compensate, new kaolin was added every 2.5 h, but clearing rates were sufficiently fast that kaolin concentrations fluctuated to some extent. Clearing of the drilling effluent particulates was much less effective so that new drilling effluent was added only when the seawater was changed. All solutions were changed every 8 hr.

At 2 days and 5 days the oysters were opened and the gill tissue removed and immediately frozen on dry ice. The oyster gill was homogenized at 4°C. The supernatant remaining after centrifugation was brought up to 10% TCA with 50% TCA to remove the proteins and nucleic acids. After centrifugation the supernatant was extracted six times with ether to remove the TCA and freeze-dried. The lyophilate was taken up in 0.1 N HCl and analyzed on an Aminco Aminolyzer using a sodium citrate buffer system with orthophthaldialdehyde (OPA) as the detecting compound. Proline and cysteine, which do not react with OPA, were not measured. Some cysteine may be included in the cysteic acid values since oxidation was not controlled. Serine, glutamine and asparagine coeluted in our system. Since the sum of these three was small, no acid hydrolysis step was used to separate the three. They are reported as the sum based on serine as a control. Threonine, isoleucine, leucine, phenylalanine, tyrosine and lysine were present in trace amounts relative to the others and are not reported here.

Protein was estimated by incubating the pellet derived from the first homogenization and the TCA pellet in a solution of 10% SDS; 0.8 N NaOH: H_2O (1:1:2) for 30 min and running a Lowry protein assay as modified by Peterson (1977) on the soluble protein fraction. Amino acid results are reported as μmoles amino acid per mg protein, thus assuming that no significant changes in protein occurred over the 2- and 5-day experimental period.

RESULTS

Means and standard deviations for the amino acids of control and treated oysters are found in Table 1. Statistically significant changes for specific amino acids are recorded in Tables 1–3. Standard deviations were particularly high for taurine which normally totalled over half of the entire free amino acid pool in oyster gill. This high variability in taurine biased statistical tests. Thus, *t*-tests for difference in means are reported for both the total amino acid pool and for the total pool without taurine (Table 1–3).

Estaurine invertebrates normally respond to hyperosmotic stress with a rise in the concentrations of non-essential amino acids such as glycine and alanine. In oyster gill tissue exposed to a 12‰ increase (from 26‰ to 38‰) for 2 days, glycine, alanine and β-alanine increased significantly. Glycine and alanine accounted for over 95% of the total increase in pool size when taurine is not considered (total less taurine, Table 1). Similar results have been reported for oyster adductor muscle (Lynch & Wood, 1966; Shumway & Gabbott, 1977), and in other bivalves (e.g. Virkar & Webb, 1970). At 5 days glycine, alanine and β-alanine were still significantly above control levels as was the total FAA pool without taurine. Glycine, however, was significantly below its 2-day value, but, because of a non-significant increase in cysteic acid, the total pool size was not significantly different from the 2-day value. Therefore, although osmotically-induced changes in individual amino acids continued to occur for at least 5 days, the FAA pool's response to counteract hyperosmotic stress in oyster gill was nearly complete at the end of 2 days.

Anaerobic metabolism in molluscs is normally manifested in the FAA pool by a decrease in aspartic acid and an increase in alanine (de Zwaan *et al.*, 1976; Collicutt & Hochachka, 1977; Hochachka, 1980). As expected, in oyster gill tissue, there was a significant increase in alanine and a significant decrease in aspartic acid at 2 days. In addition, glycine and β-alanine increased significantly. At 5 days, alanine remained significantly above and aspartic acid below control values, but the mean values were nearly half of those noted at 2 days. Interestingly, at 5 days, taurine and glutamic acid were significantly below control values. Moreover, taurine and glutamic acid, as well as glycine were significantly lower at 5 days than 2 days. In addition, the entire FAA pool was depressed significantly when compared to 5-day controls or 2-day anoxic oysters.

The effect of turbidity as a stress on the FAA pool has not been previously studied. After 2 days at a particulate load about three times that noted by Loosanoff (1962) to depress filtering rates, little effect on the FAA pool could be noted. β-alanine increased significantly and there was an apparent, but non-significant, increase in cysteic acid. During the first 2 days, clearing rates were high; the kaolin was removed from the water rapidly. Even with periodic replacement, oysters were able to clear the water sufficiently fast to reduce the stress significantly during a portion of the experimental period. As noted by Loosanoff (1962), oysters appear to recover rapidly from short-term bouts with high turbidity. Thus, it is likely that the changes in the FAA pool would have been

Table 1. The concentration of amino acids in the FAA pool in control and treated oysters at two and five days. The concentration means are in μmoles amino acid/mg protein

Amino Acid	Control		Increased Salinity		Anoxia		Drilling Effluent		Kaolin	
	Two Day (n=4)	Five Day (n=5)	Two Day (n=4)	Five Day (n=4)	Two Day (n=5)	Five Day (n=4)	Two Day (n=5)	Five Day (n=5)	Two Day (n=5)	Five Day (n=4)
Cysteic Acid *C_2D_2; S_2D_2; *S_2K_2; S_5A_5; *S_2S_5; *D_2D_5	2.37 (0.72)	3.22 (1.54)	1.88 (0.81)	3.43 (1.07)	3.20 (2.07)	1.60 (0.80)	3.84 (1.23)	1.94 (1.01)	4.19 (1.88)	2.56 (1.05)
Taurine C_5A_5; C_5D_5; C_5K_5; S_5A_5; S_5D_5; S_5K_5; *S_2K_2 *A_2A_5; D_5D_5; K_5K_5	16.37 (3.39)	18.84 (3.74)	14.31 (3.64)	19.41 (5.70)	28.41 (15.49)	12.02 (3.93)	17.82 (3.16)	11.17 (2.35)	20.54 (5.63)	11.88 (2.84)
Aspartic Acid *C_2A_2; A_2D_2; A_2K_2; C_5A_5; C_5D_5; C_5K_5; S_5A_5; *S_5D_5; *A_5K_5; D_5D_5; K_5K_5	2.59 (0.72)	2.08 (0.28)	2.14 (0.73)	1.94 (0.77)	1.48 (0.81)	0.74 (0.18)	2.65 (0.61)	1.06 (0.41)	2.89 (0.77)	1.33 (0.51)
Serine, Glutamine, Asparagine *C_5D_5; S_5A_5; S_5D_5; S_5K_5; A_2A_5; D_2D_5; K_2K_5	0.53 (0.13)	0.49 (0.18)	0.65 (0.09)	0.65 (0.16)	0.93 (0.65)	0.25 (0.14)	0.70 (0.16)	0.33 (0.05)	0.65 (0.19)	0.29 (0.18)
Glutamic Acid C_2D_2; *S_2D_2; C_5A_5; C_5D_5; *C_5K_5; S_5A_5; S_5D_5; *S_5K_5; A_2A_5; D_2D_5; *K_2K_5	1.30 (0.19)	1.42 (0.27)	1.52 (0.35)	1.41 (0.11)	1.85 (0.78)	0.83 (0.30)	2.03 (0.40)	1.04 (0.20)	1.65 (0.51)	1.09 (0.24)
Glycine C_2S_2; *C_2A_2; *S_2A_2; S_2D_2; S_2K_2; *A_2D_2; *A_2K_2; C_5S_5; C_5D_5; C_5K_5; S_5A_5; S_5D_5; S_5K_5 D_2D_5; *K_2K_5; A_5A_5; S_2S_5	1.29 (0.29)	1.36 (0.27)	3.56 (0.69)	2.25 (0.29)	2.25 (0.94)	0.98 (0.35)	1.28 (0.37)	0.74 (0.17)	1.30 (0.51)	0.72 (0.34)
Alanine C_2S_2; *C_2A_2; C_2D_2; S_2D_2; S_2K_2; *A_2D_2; A_2K_2; D_2K_2; C_5S_5; C_5A_5; *C_5K_5; S_5A_5; S_5D_5; S_5K_5; A_5D_5; A_5K_5; D_5K_5; D_2D_5; K_2K_5	1.30 (0.27)	1.27 (0.26)	4.76 (1.19)	3.65 (0.85)	4.27 (2.63)	2.22 (0.69)	2.08 (0.38)	1.45 (0.32)	1.50 (0.36)	0.90 (0.31)
β-alanine C_2S_2; C_2A_2; C_2D_2; C_2K_2; S_2A_2; S_2K_2; C_5S_5; S_5A_5; S_5D_5; S_5K_5; *D_2D_5	0.25 (0.13)	0.40 (0.13)	0.80 (0.10)	0.82 (0.18)	0.51 (0.18)	0.45 (0.13)	0.59 (0.29)	0.34 (0.08)	0.40 (0.09)	0.35 (0.18)
Total C_5A_5; C_5D_5; C_5K_5; S_5A_5; S_5K_5; S_5D_5 D_2D_5; K_2K_5; A_2A_5	26.63 (4.82)	29.21 (3.39)	30.39 (6.70)	33.60 (7.19)	43.33 (23.11)	19.27 (5.40)	31.33 (4.80)	17.85 (1.46)	33.43 (9.25)	19.27 (5.30)
Total less taurine C_2S_2; C_2D_2; C_5S_5; C_5A_5; C_5D_5; C_5K_5; S_5A_5; S_5D_5; S_5K_5; *A_2A_5; D_2D_5; K_2K_5	10.26 (1.44)	10.37 (1.65)	16.08 (3.26)	14.19 (2.03)	14.92 (7.86)	7.25 (1.62)	13.51 (2.40)	6.68 (1.19)	12.88 (3.89)	7.39 (2.60)

Standard deviations are in parentheses. Serine–glutamine–aspargine values are based on a serine standard. Comparisons of mean values (using Student's *t*-test) in which significant differences occurred ($\alpha = 0.05$ or, when designated by *, at the $\alpha = 0.10$ level) appear below each amino acid as paired symbols designating the means compared. Abbreviations: C_2, control—2 day; C_5, control—5 day; S_2.S_5, increased salinity; A_2, A_5, anoxia; D_2.D_5, drilling effluents, K_2.K_5, kaolin.

more pronounced if kaolin had been present continuously in high concentrations. After the first 2 days, observations indicated that clearing rates had decreased substantially. By five days, kaolin-stressed oysters showed significant decreases in taurine, aspartic acid, glutamic acid, glycine and alanine as well as the entire FAA pool as compared to the 5-day controls. Cysteic acid also appeared to drop, but the

Table 2. Pair-wise comparisons between all treatments with amino acids whose means were significantly different at the $\alpha = 0.05$, or if designated by *, at the $\alpha = 0.10$ levels at 2 or at 5 days listed below each pair

Oysters-Two Days

Control vs. Salinity	Control vs. Anoxia	Control vs. Drilling Effluents	Control vs. Kaolin	Salinity vs. Anoxia	Salinity vs. Drilling Effluents	Salinity vs. Kaolin	Anoxia vs. Drilling Effluents	Anoxia vs. Kaolin	Drilling Effluents vs. Kaolin
		↑*cysteic acid			↑ cysteic acid	↑*cysteic acid			
						↑*taurine			
	↓*aspartic acid						↑ aspartic acid	↑ aspartic acid	
		↑ glutamic acid			↑*glutamic acid				
↑glycine	↑*glycine			↓*glycine	↓ glycine	↓ glycine	↓*glycine	↓*glycine	
↑alanine	↑*alanine	↑ alanine			↓ alanine	↓ alanine	↓*alanine	↓ alanine	↓alanine
↑β-alanine	↑ β-alanine	↑β-alanine	↑β-alanine	↓ β-alanine		↓ β-alanine			
↑Total less taurine		↑Total less taurine							

Oysters-Five Days

				↓ cysteic acid					
	↓ taurine	↓ taurine	↓ taurine	↓ taurine	↓ taurine	↓ taurine			
	↓ aspartic acid	↓ aspartic acid	↓ aspartic acid	↓ aspartic acid	↓*aspartic acid			↑*aspartic acid	
		↓*serine +		↓ serine +	↓ serine +	↓ serine +			
	↓ glutamic acid	↓ glutamic acid	↓*glutamic acid	↓ glutamic acid	↓ glutamic acid	↓*glutamic acid			
↑glycine		↓ glycine	↓ glycine	↓ glycine	↓ glycine	↓ glycine			
↑alanine	↑ alanine		↓*alanine	↓ alanine	↓ alanine	↓ alanine	↓ alanine	↓ alanine	↓alanine
↑β-alanine				↓ β-alanine	↓ β-alanine	↓ β-alanine			
	↑ Total	↑ Total	Total	↑ Total	↑ Total	↑ Total			
↑Total less taurine	↓ Total less taurine	↑Total less taurine	↑ Total less taurine	↑ Total less taurine	↑ Total less taurine	↑ Total less taurine			

Arrows indicate whether the amino acid concentration in the second (or lower) treatment was increased (↑) or decreased (↓) over the first (or upper) treatment of each pair. Serine† includes serine, glutamine and asparagine with serine used as a control.

Table 3. Comparisons between 2-day and 5-day oysters for each treatment with amino acids whose means were significantly different at the $\alpha = 0.05$, or if designated by *, at the $\alpha = 0.10$ level listed below each pair

Control-2 day vs. Control-5 day	Salinity-2 day vs. Salinity-5 day	Anoxia-2 day vs. Anoxia-5 day	Drilling Effluents 2 day vs. Drilling Effluents 5 day	Kaolin-2 day vs. Kaolin-5 day
None	↑*cysteic acid		↓*cysteic acid	
		↓*taurine	↓ taurine	↓ taurine
			↓ aspartic acid	↓ aspartic acid
		↓*serine +	↓ serine +	↓ serine +
		↓ glutamic acid	↓ glutamic acid	↓*glutamic acid
	↓ glycine	↓ glycine	↓ glycine	↓*glycine
			↓ alanine	↓ alanine
			↓*β-alanine	
		↓*Total	↓ Total	↓ Total
		↓*Total less taurine	↓ Total less taurine	↓ Total less taurine

Serine† includes serine, glutamine and asparagine with serine used as a control.

value was not statistically different from the controls. In addition, all amino acids except cysteic acid dropped significantly in the 5-day treatments when compared to the 2-day treatments. The total FAA pool was also significantly lower.

Drilling effluents are an important pollutant associated with drilling operations. The drilling fluid used here was a high lignosulfonate mud. Oysters exposed to drilling effluents are exposed to turbidity stress as well as the presence of increased levels of heavy metals, such as chromium, which are known to be toxic at high concentrations (e.g. Calabrese *et al.*, 1973). Therefore, drilling effluents are an example of a multiple stressing agent. Observations of drilling effluent-treated oysters suggested that clearing rates were substantially less than in kaolin-treated oysters. This was probably due to an increased incidence of valve closure in drilling effluent-exposed oysters. Thus, self-induced anoxia may have been an additional stress. At 2 days, cysteic acid, glutamic acid, alanine and β-alanine were significantly above control levels in drilling effluent-treated oysters. The FAA pool minus taurine was also significantly increased. Alanine levels, though significantly above control levels were significantly below levels found in anoxia-stressed oysters. Cysteic acid levels were approximately equivalent to levels found in kaolin-treated oysters. At 5 days, taurine, aspartic acid, glutamic acid, glycine and the entire FAA pool were below control levels. All measured amino acids had dropped significantly from levels measured in 2-day drilling effluent-treated oysters. This drop at five days resembled drops also noted in kaolin-treated and anoxia-treated oysters.

DISCUSSION

Stress induced changes in the FAA pool can be caused by at least three phenomena. In some cases, specific sets of metabolic pathways are present for meeting specific needs such as responding to salinity change or to anoxia. If two occur together, some additive or synergistic effect may be found. In other cases, the entire amino acid pool may change, with individual amino acids being affected more or less equally so that the proportions present within the FAA pool shift relatively little. Finally, unnatural stresses for which no prior adaptive mechanism is present may cause a multiple stress response by turning on more than one set of metabolic pathways normally used to counter natural stress. These may affect the FAA pool in an additive or synergistic way.

Specific pathways are probably present for most stresses normally and naturally encountered by marine organisms. For example, anoxia induced the expected changes in the oyster's metabolism which manifest themselves in the FAA pool as a decrease in aspartic acid concentration, and increases in glycine and alanine concentration. The glycine increase suggests that strombine formation may be important in oyster gill tissue during anoxia (Hochachka, 1980; Fields & Quinn, 1981). Salinity stress also induces glycine and alanine formation in oyster gill tissue, but it is unlikely that the pathways and control systems induced are the same (e.g. Baginski & Pierce, 1978).

β-alanine also increased significantly over controls. This increase was much more dramatic in the salinity-stressed oyster than in other treatments although statistically significant increases in the other treatments were found. In addition, at 5 days the β-alanine value in salinity-treated oysters remained high, whereas, all others had returned to control levels. Therefore, the β-alanine increase seems to be predominantly a result of high salinity stress. A similar finding has been reported by Kasschau & Chen (1978) and Howard & Kasschau (1980) in the anemone *Bunodosoma cavernata* where β-alanine concentrations were found to be directly related to salinity increase in both laboratory acclimated animals and in field studies. The increase in β-alanine, although three times control levels, nevertheless plays a rather small role in the FAA pool's response to osmotic stress (9%). Solute increase is predominantly caused by increases in glycine (39%) and alanine (59%) concentration. Thus it is unlikely that the β-alanine increase can be explained simply on the necessity of increasing solute concentration, although salinity induction of β-alanine synthesis is beyond question.

Although the effects of salinity plus anoxia on the FAA pool are well known, the FAA pool probably responds to a variety of other stressing agents with amino acid-specific changes (Kasschau, 1975b; Kasschau *et al.*, 1980). The possible effects of turbidity as a stress have not been studied previously, although Loosanoff & Tommers (1948), Loosanoff (1962) and others have documented behavioral and physiological changes in oysters exposed to turbidity primarily as it relates to interference with filtering capability. In addition, Low *et al.* (1980) have determined that kaolin, the agent causing turbidity in this study, interferes with amino acid transport and protein metabolism in rabbit lung tissue. The most prominent change in the kaolin-stressed oysters was an increase in cysteic acid level. Cysteic acid and taurine levels were highly variable in comparison to other amino acids. This variability prevented significant *t*-tests in most cases. Nevertheless, it is interesting to note that the drilling effluent treated oysters which also were exposed to turbidity were the only ones to have a statistically significant increase in cysteic acid at 2 days. Thus cysteic acid increases were only noted in 2-day oysters affected by turbidity.

Cysteic acid is intimitately involved in sulfur metabolism (Abbott & Awapara, 1960; Jacobsen & Smith, 1968; Allen & Garrett, 1971) and particularly taurine formation. A comparison of cysteic acid and taurine levels in all of the five treatments at 2 days and 5 days reveals that increases or decreases in taurine concentration at 5 days were always accompanied by a cysteic acid change in the same direction within treatments. For example, both taurine and cysteic acid increased at day 5 in the salinity-treated oysters, whereas, both decreased in kaolin-treated ones relative to their respective 2-day levels.

Increased taurine formation could be the explanation for the cysteic acid increase in kaolin and drilling effluent-treated oysters at day 2 although the significance of such formation for meeting turbidity stress is unclear. Alternatively, since most invertebrate mucus is known to have a high sulfate anion content (Hunt, 1970; Pancake & Karnovsky, 1971; Rahemtulla &

Løvtrup, 1975), and since an increase in mucus secretion is almost certainly required to combat turbidity, an increase in sulfur metabolism in general might have occurred. In this case since phosphoadenosinephosphosulfate (PAPS) can be a precursor for cysteic acid, (Jacobsen & Smith, 1968; Allen & Garrett, 1971), increased sulfate activation by PAPS formation during mucus synthesis may also generate some cysteic acid. Whatever the reason, cysteic acid seems to be an important compound in the FAA pool's response to high turbidity stress.

Besides specific responses of certain individual amino acids to specific stressing agents, some stress responses involve changes in the entire amino acid pool with individual amino acids being affected more or less equally so that the proportions present within the FAA pool shift relatively little. Protein may be broken down, for example, increasing the level of amino acids in the FAA pool (Riley, 1980; Mommsen *et al.*, 1980). In other cases, such as the response to hypoosmotic stress in some invertebrates, a general loss of most or all amino acids may occur (e.g. Amende & Pierce, 1980a).

The FAA pool in kaolin, drilling effluent, and anoxia stressed oysters at 5 days showed a dramatic decrease in nearly all measured amino acids when compared to either the 5-day controls or the 2-day treatment groups. Interestingly, whatever the cause of the decrease on day 5, it appears to have operated on all of the amino acids more or less equally. It is unlikely that a stress specific response equivalent to the responses to salinity change or anoxia is responsible since the response occurred in both kaolin and anoxia-stressed oysters. The anoxia-stressed oysters received no turbidity stress and could filter normally since bubbled nitrogen was used to produce anoxia. The kaolin-stressed oysters had sufficient oxygen. Thus, the FAA decrease noted at five days was triggered by two ostensibly independent stressing agents. A similar response was not apparent in the salinity-stressed oysters. It appears likely that a general reduction of the FAA pool relative to the soluble protein concentration has occurred in drilling effluent, kaolin-, and anoxia-treated oysters from day 2 to day 5.

If one assumes that an increase in protein concentration is not the answer, and this is suggested since stress normally accelerates protein catabolism in animals, then two possible explanations exist. (1) Many of the amino acids affected, such as glutamic acid, glycine and alanine, feed into the glycolytic pathway or the TCA cycle (e.g. Goldberg & Chang, 1978; Chang & Goldberg, 1978) and thus a general draw on the FAA pool to meet the energy requirements of combating these stresses might be indicated (e.g. Riley, 1976, 1980). The reduction is also quite clear in taurine and, to a lesser extent, cysteic acid, however. Explaining these latter changes based on amino acid breakdown for ATP production requires pathways not well known in marine organisms (King *et al.*, 1980; Allen & Garrett, 1971). (2) Although these pathways might be present, the data might also suggest a simple and general loss of FAA from the gills, perhaps due to some changes in the tissue's ability to prevent FAA loss across the outer cell membrane. Amende & Pierce (1980b) have shown that changes in ATP availability can change the flux of FAA across membranes, for example. A change in membrane ultrastructure has been associated with hypo-osmotic stress and increased membrane permeability to amino acids (Amende & Pierce, 1980c). Changes in membrane structure have also been associated with exposure to kaolin (Low *et al.*, 1980) and to heavy metals (Engel & Fowler, 1979). Such phenomena might be expected in the gill tissue of kaolin-, anoxia- and drilling effluent-treated oysters in this study. Regardless of the explanation, this amino acid loss was the most dramatic change found at day 5. The total FAA pool decreased by nearly 50% (as did the concentrations of nearly all the individual amino acids) in all three cases whereas no significant change was noticed in the controls and a clear increase occurred in salinity-stressed oysters.

Unnatural stresses for which no prior adaptive mechanism is present may result in the functioning of more than one set of metabolic pathways providing a unique suite of FAA concentrations or modifying a second natural response in an unexpected way (Squires *et al.*, 1979; Roesijadi *et al.*, 1976). Drilling effluents are an example of a stressing agent which induces more than a single set of metabolic responses. Oysters exposed to drilling effluents are exposed to anoxia, turbidity, and the presence of various noxious components such as heavy metals. The response by the FAA pool appears to involve components of all three. Thus, the cysteic acid increase on day 2 in drilling effluent-treated oysters was probably in response to the turbidity stress. A small but significant increase in alanine may well have been associated with periods of self-induced anoxia. Alanine levels for these animals fell intermediate between control and permanently anoxic oysters in this study, although the aspartic acid decrease noted in permanently anoxic oysters was not present. The glutamic acid increase, although possibly related to anoxia, has been noted as a consistent response to heavy metal pollution in anemones by Kasschau *et al.* (1980), suggesting that this may be an effect of the heavy metal concentrations in the drilling effluent. The experiments reported here do not prove that the amino acid changes noted were induced as suggested above, of course. Nevertheless, they suggest that the overall FAA response to exposure to drilling effluents might be subdivided into components related to the various stresses actually impinging on the organism.

A number of metabolic indices of stress have been proposed to monitor health in various species of invertebrates (e.g. Bayne *et al.*, 1979; Jeffries, 1972). Amino acids have been suggested as a potentially valuable tool since they are easily quantitated and distinct changes occur in response to a variety of stress conditions (Sansone *et al.*, 1978; Riley, 1980; and others referenced herein). The results reported here strongly support this basic concept although much work must be done before it can be usefully applied to any species. In this study, a statistical analysis comparing the mean values of the amino acids measured between all treatments at 2 and 5 days revealed no case where some changes were not specific for the time and treatment used (except the 2- and 5-day controls which were identical) (Tables 2 and 3). Thus, from any given amino acid pattern found in this study, the specific treatment and time

could have been accurately predicted providing the suite of possible treatments and times used was known.

This set of experiments suggests that many stresses may be distinguished by specific changes in the FAA pool and that such changes in the FAA pool may be related to and be indicative of the overall health of these organisms. Many other indices of stress or health have been suggested, such as variations in enzyme activity, in calcification rate or in oxygen utilization. Use of the FAA pool carries with it the particularly important assets that (1) many different pathways and interactions are potentially possible, more than one of which can operate at any given time giving a multitude of potential FAA response patterns and (2) a significant number of different compounds can be measured rapidly and easily. In these experiments, the FAA pool's response to the stress-inducing agents present could easily be distinguished one from another without a prior knowledge of the specific stress involved. This suggests that changes in the FAA pool may prove to be an extremely valuable clue to the health and the causes of stress in marine organisms.

Acknowledgements—We thank Dr. Thomas Bright for his help and encouragement. We also thank James Kendall who helped collect the oysters, Jim Parrack whose suggestions improved the manuscript considerably, and Becky Covington who typed the manuscript. The drilling effluent was furnished by EPA–Gulf Breeze Research Laboratory.

This project has been financed entirely with Federal funds from the Environmental Protection Agency under Grant No. R-806793-01. The contents do not necessarily reflect the views and policies of the Environmental Protection Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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